"LATENT" HEALTHY HUMAN SERUM AUTOANTIBODIES CROSS-REACTING WITH DNA AND BACTERIAL LIPOPOLYSACCHARIDES

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Autoantibodies to native DNA play an important role in the pathogenesis of systemic lupus erythematosus (SLE). Autoantibodies of this kind, whether obtained by hybridoma technology or isolated from the blood serum of SLE patients or mice with lupuslike diseases, basically do not possess narrow specificity. They can interact not only with DNA, but also with synthetic polynucleotides, cardiolipin, and other phospholipids [12, 15], with cytoskeletal proteins [3, 4], and with cell membranes [13]. Recent investigations have shown that monoclonal antibodies to DNA can interact with lipopolysaccharides (LPS) of widely distributed bacteria, and antibodies to LPS can react with DNA [5, 9]. During polyclonal stimulation of B lymphocytes by LPS, antibodies to DNA may appear [6, 8]. On the basis of these findings the possible evolutionary connection at the genetic level between antibodies to LPS and antibodies to DNA has been postulated. The writers previously found antibodies to DNA in healthy human blood serum in a latent state (evidently, in the composition of immune complexes) [2]. These antibodies belong to the IgG class and can be demonstrated by ion-exchange chromatography on QAE-Sephadex A-50. After treatment in this way the antibodies become capable of reacting with native and denatured DNA, with certain polynucleotides, and with dextran sulfate and they differ only a little in polyreactivity from the known panel of monoclonal antibodies to DNA. In the investigation described below the ability of "latent" antibodies to DNA from healthy human blood serum to interact with bacterial LPS was studied.

EXPERIMENTAL METHOD

LPS of widely distributed bacteria were used, namely: *Escherichia coli* (from "Sigma"), *Pseudomonas aeruginosa, Shigella boydii,* and *Salmonella typhi* (supplied the staff of the laboratory of microbial antigen chemistry, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR Moscow). IgG were isolated from serum obtained from healthy blood donors by the standard method: triple reprecipitation by ammonium sulfate followed by chromatography on DEAE-cellulose in 0.01 M potassium-phosphate buffer, pH 8.0. Chromatography of IgG on QAE-Sephadex was carried out in 0.01 M potassium-phosphate buffer, pH 7.2, as described previously [2]. The IgG fraction not adsorbed on the QAE-Sephadex was described as A1, the fraction bound with the exchange resin and eluted from it by 0.5 M sodium chloride solution was described as A2. Labeled DNA was isolated from *E. coli* cells grown in the presence of tritium-labeled thymidine. The ability of IgG to interact with tritiated DNA was detected by radioimmunoassay [2], and with LPS by inhibition of the direct reaction of IgG with ^{3}H-DNA, using LPS as inhibitor. The results were expressed as percentage inhibition of the reaction of IgG with labeled DNA.

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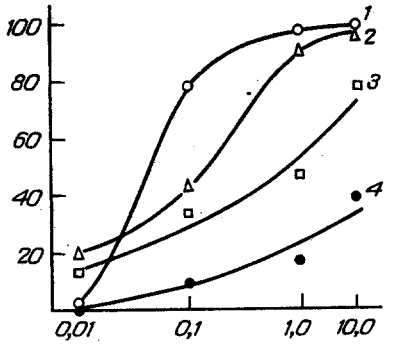

Fig 1. Inhibition of interaction of A1 fraction of IgG with labeled DNA by LPS from different species of bacteria. 1) *Ps. aeruginosa*; 2) *Sh. boydii*; 3) *S. typhi*; 4) *E. coli*. Abscissa, concentration of LPS (inhibitor) (in μg/ml, logarithmic scale); ordinate, degree of inhibition (in %).

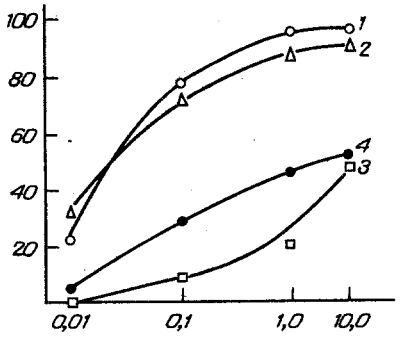

Fig. 2. Inhibition of interaction of A2 fraction of IgG with labeled DNA by LPS of different species of bacteria. Legend as to Fig. 1.

EXPERIMENTAL RESULTS

Data on interaction between the A1 fraction of IgG and the various LPS are given in Fig. 1. They show that IgG reacting with DNA also interact with LPS. Maximal inhibition of binding of IgG with ^{3}H-DNA was observed when LPS of *Ps. aeruginosa* and *Sh. boydii* was used as inhibitor LPS from *S. typhi* and *E. coli* inhibited this reaction by a lesser degree. The effect of inhibition disappeared when the inhibitor was used in a concentration of 10 ng/ml.

The results indicate that the total IgG fraction from healthy human blood serum contains immunoglobulins which, after activation on QAE-Sephadex, can cross react both with DNA and with bacterial LPS. Considering that these antibodies also react with poly(I) and poly(G) [2], which closely resemble in their conformation the double helix of DNA, and with cardiolipin, which, like DNA, contains a phosphoric acid residue in its composition [1], it can be tentatively suggested that the antigenic determinants in LPS for these antibodies may be charged groups, including phosphoric acid residues.

Recent data indicate that the genes for antibodies to DNA and to LPS may be very close nucleotide sequences, and in turn, they may arise from embryonic immunoglobulin genes [11]. It has also been suggested that bacterial agents may be the cause of autoimmune diseases. An indication of this is given by cross reactions of antibodies to DNA and to LPS [14], and the ability of blood sera from patients with bacterial infections to bind native and denatured DNA [10]. Another possibility is that genes of antibodies to DNA and LPS arise from common ancestral genes, and differences in the specificity of antibodies may be the result of point mutations in the gene, leading to amino acid substitutions in immunoglobulin. In particular, it was shown in [7] that monoclonal S107 antibodies to phosphocholine, after a point mutation in the V(H) gene, can no longer bind bacterial antigen, but they begin to react with native DNA and with cardiolipin.

It has been suggested that such antibodies, cross-reacting both with LPS and with the individual's own antigens, may be the first line of defense against foreign agents, and in the case of any disturbances in the immune system, they may induce autoimmune reactions and cause the development of autoimmune diseases. The protective function described above can perhaps be performed by the latent polyspecific autoantibodies which we found in healthy human blood serum.

LITERATURE CITED

1. V. A. Saenko, G. M. Rott, and A. M. Poverennyi, Byull. Éksp. Biol. Med., No. 2, 217 (1989).
2. N. I. Sulaeva, I. V. Lekakh, and A. M. Poverennyi, Biokhimiya, **51**, 574 (1986).
3. J. Andre-Schwartz, S. K. Datta, F. Shoenfeld, et al., J. Clin. Immunol. Immunopath., **31**, 261 (1984).
4. E. Cairns, R. Komar, and D. A. Bell, Arthr. Rheum., **29**, 1351 (1986).
5. P. Carrol, D. Stafford, R. S. Schwartz, and B. D. Stollar, J. Immunol., **135**, 1086 (1985).
6. O. Dar, M. R. Solomon, M. H. Seifert, and D. A. Isenberg, Clin. Exp. Immunol., **73**, 430 (1988).
7. B. Diamond and M. Scharff, Proc. Nat. Acad. Sci. USA, **81**, 5841 (1984).
8. A. El-Roeiy, W. L. Cross, J. Ludemann, et al., Immunol. Lett., **12**, 313 (1986).
9. A. El-Roeiy, O. Sela, D. A. Isenberg, et al., Clin. Exp. Immunol., **67**, 507 (1987).
10. D. A. Isenberg, P. Maddison, G. Swana, et al., Clin. Exp. Immunol., **67**, 516 (1987).
11. R. Kofler, D. J. Noonan, D. E. Levy, et al., J. Exp. Med., **161**, 805 (1985).
12. E. M. Lafter, J. Rauch, C. J. Andrzejewski, et al., J. Exp. Med., **153**, 897 (1981).
13. E. Muso and L. Jacob, Clin. Immunol. Immunopath., **42**, 370 (1987).
14. Y. Naparstek, D. Duggan, A. Schattner, et al., J. Exp. Med., **161**, 1525 (1985).
15. J. Rauch, H. Tannenbaum, B. D. Stollar, and R. S. Schwartz, Eur. J. Immunol., **14**, 529 (1984).